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| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 09/058,323 | 04/09/1998 | BEREND HOUWEN | 10690/101683 | 7347 |

7590 11/19/2003

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| EXAMINER | |
|---|---|
| GABEL, GAILENE | |
| ART UNIT | PAPER NUMBER |
| 1641 | |

DATE MAILED: 11/19/2003

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Please find below and/or attached an Office communication concerning this application or proceeding.

| Office Action Summary | Application No. | Applicant(s) | |
|---|---|---|---|
| | 09/058,323 | HOUWEN ET AL. | |
| | Examiner | Art Unit | |
| | Gailene R. Gabel | 1641 | |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☒ Responsive to communication(s) filed on *14 August 2003*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4)☒ Claim(s) *1-13* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-9 and 13* is/are rejected.

7)☒ Claim(s) *10-12* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

**Application Papers**

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. §§ 119 and 120**

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

13)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application) since a specific reference was included in the first sentence of the specification or in an Application Data Sheet. 37 CFR 1.78.

a) ☐ The translation of the foreign language provisional application has been received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121 since a specific reference was included in the first sentence of the specification or in an Application Data Sheet. 37 CFR 1.78.

**Attachment(s)**

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

## DETAILED ACTION

### *Applicant's Response*

1. Applicant's response filed 8/14/03 in Paper No. 31 is acknowledged. Currently, claims 1-13 are pending and are under examination.

### ***Rejections Withdrawn***

### *Claim Rejections - 35 USC § 103*

2. In light of Applicant's argument, the rejection of claims 10-12 under 35 U.S.C. 103(a) as being unpatentable over Kim et al. (US 5,648,225) in view of Loken et al. (US 5,047,321) as applied to claims 1-3 and 5-9, and in further view of Inami et al. (US 5,298,426) is hereby, withdrawn.

### ***Rejections Maintained***

### *Claim Rejections - 35 USC § 103*

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein

were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

3. Claims 1-3 and 5-9 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Kim et al. (US 5,648,225) in view of Loken et al. (US 5,047,321).

Kim et al. disclose methods for 1) discriminating and counting erythroblasts, 2) determining classes of leucocytes, and 3) immunophenotyping lymphocytes, using a multipurpose reagent system (see Abstract). The method comprises adding the multipurpose reagent system to an anticoagulated blood sample, incubating the mixture, and subjecting the mixture to flow cytometric analysis (see column 6, lines 6-17). Specifically, the reagent system includes proper concentrations of aldehydes, non-quaternary mono-ammonium salt, and buffer to lyse the nucleated and non-nucleated red cells while maintaining the integrity of the fixed white blood cells (see column 3, lines 60-65). In addition, it includes a buffer that maintains the pH at 5.5-7.5 (see column 7, lines 1-16). Specifically Kim et al. disclose that incubating the blood sample with the reagent system at slightly elevated temperatures, effectively preserves white cell membrane integrity and retains antigenicity of lymphocyte surface antigens (see column 7, lines 37-41). Certain high concentrations of ingredients to lyse nRBC's are damaging to integrity of white cells and therefore requiring a rapid quenching to the lytic activity of the reagent (see column7, lines 42-47). In addition, the reagent further

comprises a nucleotide fluorescent dye, ethidium homodimer, which reacts with exposed nuclei of nRBCs, i.e. erythroblasts, but impenetrable to intact white cells to allow quantitative analysis of nucleated red cells (see column 8, lines 32-54). The reagent also further comprises fluorochrome-conjugated antibodies directed to leucocyte surface antigens to allow quantitative analysis and differentiation of leucocytes, i.e. anti-CD4, anti-CD8 conjugated to FITC, PE, etc. (see column 8, line 65 to column 9, line 22). Electronic signals from scattered light collected from different angles and fluorescence intensities are plotted as two dimensional plots (see column 6, lines 31-46 and also Figure 3).

Kim et al. is silent in teaching that the multipurpose reagent is used in simultaneously analyzing hematological samples to 1) discriminate erythroblasts by detecting nucleotide fluorescent signal and 2) determine leucocyte classes by detecting signal from labeled anti-leucocyte antibodies that bound to cell surface antigens in the leucocytes using multiparameter flow cytometric analysis.

Loken et al. disclose combining a whole blood sample with at least two nucleotide fluorescent dyes (RNA dye or DNA dye) and at least one fluorescent labeled antibody specific for cell surface antigen. The dyes independently and differentially assess different characteristics of nucleated cells in the sample and simultaneously, the fluorescent labeled antibody specific for cell surface antigens differentially assesses leucocytic cells of different lineages (see column 4, lines 27-40). Each of the dyes and fluorescent labeled antibody, i.e. phycoerythrin (PE) is excitable at the same wavelength and has a peak emission spectra that is distinguishable from the others (see column 5,

lines 16-20). The fluorescence intensity and light scatter of the labeled cells in the hematologic mixture is simultaneously measured and analyzed using flow cytometry.

It would have been obvious to one having ordinary skill in the art at the time the invention was made to simultaneously analyze hematological samples using flow cytometry such as taught by Loken, to 1) discriminate erythroblasts by detecting nucleotide fluorescent signal and 2) determine leucocyte classes by detecting signal from labeled anti-leucocyte antibodies that bound to cell surface antigens in the leucocytes exposed to a multipurpose reagent system such as taught by Kim, since the multipurpose reagent as taught by Kim provides lysis of nucleated red cells for reaction with nucleotide fluorescent dye, while maintaining integrity of the fixed white blood cells to thus, prevent penetration of nucleotide fluorescent dye but allow binding of fluorochrome-conjugated antibodies to leucocyte surface antigens; thus, suggesting multipurpose application of the reagent system in providing simultaneous quantitative analysis and differentiation between erythroblasts and leucocytes. It has been held that forming in one piece an article which has formerly been formed in two pieces and put together, i.e. multipurpose reagent system having both nucleotide fluorescent dye and fluorescent labeled anti-leucocyte antibodies, involves only routine skill in the art. *Howard v. Detroit Stove Works*, 150 U.S. 164 (1893).

4. Claims 4 and 13 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kim et al. (US 5,648,225) in view of Loken et al. (US 5,047,321) as applied to claims 1-3 and 5-9, and in further view of Inami et al. (US 5,298,426).

Kim et al. and Loken et al. have been discussed supra. Kim et al. and Loken et al. differ from the instant invention in failing to lyse, i.e. permeabilize the cell membranes of RBCs and nRBCs by incorporating reagents and buffers at specific pH and osmolality parameters in a two step method such as set forth in claim 4.

Inami et al. disclose a two-step method of differentiating erythroblasts from leucocytes. Inami et al. specifically disclose mixing blood with a hypotonic fluorescent dye solution capable of diffusing into erythroblasts to stain their nuclei and a buffer for maintaining the pH in the acidic range. Inami et al. further mixes the (acidic) sample mixture with a second fluid comprising a buffer that neutralizes the acidic pH in the solution to a staining pH and an osmolarity adjusting agent for adjusting the osmolarity of the solution to a value at which the shape and integrity of leucocytes are maintained (see column 2, lines 3-24 and column 4, lines 17-41). The first acidic and hypotonic fluid has a low osmolality causing erythrocytic cell lines in the sample to swell upon absorbing water causing cellular contents to leak out and nucleotide fluorescent dye (erythroblastic dye to diffuse through the cell membrane to stain their nuclei. Leucocytes do not permit the entrance of nucleotide fluorescent dye (see column 5, line 60 bridging to column 6, line 26). Inami et al. enumerates the different dyes used in the first fluid for differentiating leucocytes and erythroblasts, including propidium iodide and ethidium bromide specific for erythroblast nuclei, and appropriate concentrations thereof in column 3 of the disclosure. Inami et al. disclose that the concentration of nucleotide fluorescent dye, i.e. propidium iodide or ethidium bromide, should fall within the range of 0.003 mg/L to 10 mg/L (2.5 µg/ml to 100µg/ ml) in order to achieve optimum results (see

column 4, lines 5-16). After treatment, stained cells are measured using a flow cytometer and erythroblasts are separated from other cell groups on the resulting two-dimensional plot where erythroblasts are counted (see column 6, lines 9-12). Figure 9 shows a two-dimensional plot showing selective staining of erythroblasts with nucleotide staining dye to emit red fluorescence and to permit erythroblasts to be distributed in a separate zone from other cells so that the relative content and count can be determined. Figure 10 and 11 show two-dimensional plots for the intensity of red fluorescence versus the intensity of side-scattered light obtained for peripheral blood and bone marrow.

It would have been obvious to one of ordinary skill in the art at the time of the instant invention to incorporate the teaching of Inami in permeabilizing erythroblasts using reagent combinations having specific pH and osmolality requirements in a two step method, into the reagent system and method disclosed by Kim and modified by Loken because Kim specifically taught that integrity and antigenicity of white blood cells need to be maintained optimally during permeabilization, i.e. lysing, of the nRBC's or erythroblasts so as to provide accurate simultaneous quantitation of both populations as suggested by Loken, requiring quenching of lytic activity of the reagent because of its damaging effect to leucocytic populations and Inami specifically taught that such a procedure eliminates extreme lysing conditions for erythroblasts while maintaining the integrity and shape of WBCs to thus provide for simultaneous accurate differentiation of both erythroblast and leucocyte populations in a single sample.

***Response to Arguments***

5. Applicant's arguments filed 8/14/03 have been fully considered but they are not persuasive.

A) Applicant argues that the teaching of Kim in combination with Loken, does not render obvious the claimed invention since both references fail to provide a suggestion, motivation, or disclosure of the desirability of the combination.

Contrary to Applicant's argument, Kim explicitly provides suggestion to use the multipurpose reagent system in simultaneously "performing white cell differential analyses and quantitative analyses of nucleated red cells, i.e. erythroblasts ... on a flow cytometer" as in the method of Loken (see column 1, lines 11-17). Kim explicitly provides motivation to use the multipurpose reagent system in simultaneously "monitoring the concentration of ... leucocytes while identifying the presence of immature nucleated red cells, i.e. erythroblasts in the peripheral blood" as an important diagnostic tool for physicians (see column 1, lines 35-38). The mere capacity of the multipurpose reagent system to 1) lyse nRBCs to thus react with a nuclear stain, and 2) concurrently fix white blood cells to maintain their integrity to prevent penetration by the nuclear stain but allow for binding with fluorescent labeled antibody specific for leucocyte surface antigens, provides in and of itself, a suggestion and motivation to simultaneously achieve quantitation and differentiation of both cell lines. Absent the disclosure of item #2) aforementioned dismisses the requisite use to differentially identify leucocytes and absent the disclosure of item #1) aforementioned dismisses the requisite use to identify erythroblasts, in this one single multipurpose reagent system.

B) Applicant argues that Loken does not disclose discrimination and counting of erythroblasts from other cell groups. Applicant specifically contends that combining Loken does not motivate modification of Kim's processes or reagent to discriminate erythroblasts based on the intensity of fluorescence from a nuclear stain and labeled antibody.

In response to applicant's arguments against Kim and Loken individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). In this case, Kim discloses 1) discriminating and counting erythroblasts, 2) determining classes of leucocytes, and 3) immunophenotyping lymphocytes, using a multipurpose reagent system. The reagent comprises a nucleotide fluorescent dye which reacts with exposed nuclei of erythroblasts but impenetrable to intact white cells to allow quantitative analysis of nucleated red cells. The reagent system provides an environment to lyse nucleated red cells while maintaining the integrity of the fixed white blood cells so as to allow binding of fluorochrome-conjugated antibodies to leucocyte surface antigens to allow quantitative analysis and differentiation of leucocytes and erythroblasts. Loken was incorporated thereto, only for the teaching that a combination of nucleotide fluorescent dyes and at least one fluorescent labeled antibody specific for cell surface antigen in flow cytometric measurements, independently and differentially assess individual nucleated cell lineages in a sample. Each of the dyes and fluorescent

labeled antibody, i.e. phycoerythrin (PE) has a peak emission spectra that is distinguishable from the others. The fluorescence intensity and light scatter of the labeled cells in the hematologic mixture is simultaneously measured and analyzed using flow cytometry. Therefore, it would have been obvious to one having ordinary skill in the art at the time the invention was made to simultaneously analyze hematological samples using flow cytometry such as taught by Loken, to 1) discriminate cells such as erythroblasts by detecting nucleotide fluorescent signal and 2) determine leucocyte classes by detecting signal from labeled anti-leucocyte antibodies that bound to cell surface antigens in the leucocytes exposed to a multipurpose reagent system such as taught by Kim, since the multipurpose reagent as taught by Kim provides lysis of nucleated red cells for reaction with nucleotide fluorescent dye, while maintaining integrity of the fixed white blood cells to thus, prevent penetration of nucleotide fluorescent dye but allow binding of fluorochrome-conjugated antibodies to leucocyte surface antigens; thus, suggesting multipurpose application of the reagent system in providing simultaneous quantitative analysis and differentiation between erythroblasts and leucocytes. It has been held that forming in one piece an article which has formerly been formed in two pieces and put together, i.e. multipurpose reagent system having both nucleotide fluorescent dye and fluorescent labeled anti-leucocyte antibodies, involves only routine skill in the art. *Howard v. Detroit Stove Works*, 150 U.S. 164 (1893).

C) Applicant argues that Inami does not disclose use of antibodies as required in claim 4 wherein a first reagent is admixed with a hematologic sample having fluorescent labeled antibodies added thereto.

In response to applicant's arguments against the Inami individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). In this case, Inami is combined with the teaching of Kim and Loken to render the claimed invention obvious. Specifically, Kim provides the teaching of using a nucleotide fluorescent dye for reaction with exposed nuclei of erythroblasts and fluorochrome-conjugated antibodies to leucocyte surface antigens for discriminating and counting classes of leucocytes in a multipurpose reagent system which provides an environment to lyse nucleated red cells while maintaining the integrity of the fixed white blood cells so as to allow binding of fluorochrome-conjugated antibodies to leucocyte surface antigens to thus, allow quantitative analysis and differentiation of leucocytes and erythroblasts. Loken provides the teaching simultaneous flow cytometric analyses of nucleotide fluorescent dyes and at least one fluorescent labeled antibody specific for cell surface antigen to independently and differentially assess individual nucleated cell lineages in a sample. Inami et al. was incorporated and relied upon only for the disclosure of a two-step method of differentiating erythroblasts from leucocytes including, 1) mixing blood with a hypotonic fluorescent dye solution capable of diffusing into erythroblasts to stain their nuclei and a buffer for maintaining the pH in the acidic

range; and 2) mixing the (acidic) sample mixture with a second fluid comprising a buffer that neutralizes the acidic pH in the solution to a staining pH and an osmolarity adjusting agent for adjusting the osmolarity of the solution to a value at which the shape and integrity of leucocytes are maintained. The different dyes used in the first fluid for differentiating leucocytes and erythroblasts, include propidium iodide and ethidium bromide at concentrations within the range of 0.003 mg/L to 10 mg/L (2.5 µg/ml to 100µg/ ml) in order to achieve optimum results. Therefore, it would have been obvious to one of ordinary skill in the art at the time of the instant invention to incorporate the teaching of Inami in permeabilizing erythroblasts using reagent combinations having specific pH and osmolality requirements in a two step method, into the reagent system and method disclosed by Kim and modified by Loken because Kim specifically taught that integrity and antigenicity of white blood cells need to be maintained optimally during permeabilization, i.e. lysing, of the nRBC's or erythroblasts so as to provide accurate simultaneous quantitation of both populations as suggested by Loken, requiring quenching of lytic activity of the reagent because of its damaging effect to leucocytic populations and Inami specifically taught that such a procedure eliminates extreme lysing conditions for erythroblasts while maintaining the integrity and shape of WBCs to thus provide for simultaneous accurate differentiation of both erythroblast and leucocyte populations in a single sample.

***Allowable Subject Matter***

6. Claims 8-12 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims. Prior art of record fails to teach or fairly suggest staining and simultaneously discriminating between 1) different maturational degrees of erythroblasts using nucleotide fluorescent dye and 2) subclasses of leucocytes using fluorescent-labeled antibodies directed against leucocytic cell surface antigens in the flow cytometric method of the claimed invention.

7. **THIS ACTION IS MADE FINAL.** Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

8. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Gailene R. Gabel whose telephone number is (703)

305-0807. The examiner can normally be reached on Monday, Tuesday, and Thursday, 5:30 AM to 2:30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Long V. Le can be reached on (703) 305-3399. The fax phone number for the organization where this application or proceeding is assigned is (703) 308-4556.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 305-0169.

Gailene R. Gabel
Patent Examiner
Art Unit 1641
November 10, 2003

CHRISTOPHER L. CHIN
PRIMARY EXAMINER
GROUP 1800-1641